Journal of Food Protection, Vol. 63, No. 7, 2000, Pages 876–883

Attachment and Growth of *Salmonella* Chester on Apple Fruits and In Vivo Response of Attached Bacteria to Sanitizer Treatments†

CHING-HSING LIAO* AND GERALD M. SAPERS

U.S. Department of Agriculture, Eastern Regional Research Center, Agricultural Research Service, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

MS 99-325: Received 2 November 1999/Accepted 11 February 2000

ABSTRACT

Attachment and growth of *Salmonella* Chester on fresh-cut apple disks and in vivo response of attached bacteria to sanitizer treatments were investigated. Apple disks (14 mm in diameter and 3 to 4 mm in thickness) were immersed in a bacterial suspension that contained 8.17 log CFU/ml of *Salmonella* Chester and air dried at room temperature for 10 min. After two rinses, the population of *Salmonella* Chester retained on apple disks that contained no skin was 13 to 19% higher than that retained on disks that contained skin, indicating that *Salmonella* Chester attached more firmly to the surfaces of injured tissue than to the unbroken skin. The number of bacteria attached to the disk was not affected by the immersion time but was directly proportional to the concentration of bacteria in the suspension. The distribution of artificially inoculated *Salmonella* Chester on the surfaces of three different parts of whole fruit was determined; 94% of attached bacteria was found on the stem and calyx cavity areas and 6% on the skin of the remaining area of the fruit. Despite their acidic pH (4.1), apple disks supported the growth of *Salmonella* Chester at 20°C but not at 8°C. All four sanitizers tested in the study, including 6% hydrogen peroxide, 2% trisodium phosphate, 0.36% calcium hypochlorite, and 1.76% sodium hypochlorite, were effective in reducing the population of *Salmonella* Chester on apple disks by 1 to 2 logs. However, 5 to 13% of bacteria survived the sanitizer treatments. Hydrogen peroxide, which reduced the population of *Salmonella* Chester on skin by 3 to 4 logs and the population of bacteria on stem or calyx by 1 to 2 logs, was the most effective among the four sanitizers tested. Firm attachment of bacteria on calyx, stem, and injured tissue and partial resistance of attached bacteria to sanitizer treatments are two major obstacles to be considered when developing methods for cleaning and decontaminating apple fruits destined for juice production and fresh consumption.

Fresh apple cider contaminated with human pathogens has been implicated as the cause of four major outbreaks of foodborne illness since 1975. Two of these outbreaks involved the consumption of apple cider contaminated with *Escherichia coli* O157:H7 *(1, 6)*, and the other two involved the consumption of cider products contaminated with *Cryptosporidium parvum (6)* and *Salmonella* Typhimurium *(5)*. In addition to apple cider, several other types of fruits and vegetables, including alfalfa sprouts, watermelon, tomato, and cantaloupe *(2, 20)*, have also been found to be naturally contaminated with salmonellae and involved in disease outbreaks. Salmonellae are by far the most frequently reported cause of foodborne illness in the United States. Currently, little is known about the survival and growth characteristics of *Salmonella* on fresh produce and the sources and routes by which fruits and vegetables become contaminated.

The first step in the pathogen contamination is assumed to occur when the pathogen comes in contact with and becomes attached to food products. Food microbiologists began to investigate bacterial attachment on food products and processing equipments in the 1970s *(14, 15)*. Previous studies focused mainly on attachment of human pathogens to animal food products such as broiler chicken and beef carcasses *(4)*. Investigation of attachment of human pathogens to food plants or food plant products has not been initiated until recently. Seo and Frank *(18)* and Wachtel et al. *(21)* examined the attachment of *E. coli* O157:H7 on lettuce leaf surface by using a confocal scanning laser microscope. The purposes of this study were to (i) investigate the attachment and growth of *Salmonella* Chester on fresh-cut apple disks; (ii) determine the distribution of artificially inoculated *Salmonella* Chester on surfaces of three different parts of whole fruit, including stem, calyx, and skin; and (iii) evaluate the efficacy of four sanitizers and one detergent (Tween 80) for their potential in inactivating or removing attached bacteria on contaminated apple fruits.

MATERIALS AND METHODS

Bacterial strain, medium, and culture conditions. A *Salmonella* Chester (ATCC 11997) mutant resistant to nalidixic acid was isolated and used throughout the study. This mutant was maintained on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) that contained 20 μg/ml of nalidixic acid (designated BHIA-N) for routine cultivation. For preparation of bacterial sus-

* Author for correspondence. Tel: 215-233-6471; Fax: 215-233-6406; E-mail: cliao@arserrc.gov.

† Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

pensions, bacteria grown on BHIA-N at 37°C for 18 h were harvested and suspended in phosphate-buffered saline (PBS, pH 7.2) to make cell concentrations that ranged from 4.17 to 8.17 log CFU/ml.

Preparation of apple disks. Unwaxed apple fruits (Golden Delicious) obtained directly from the packer were cleaned and surface sanitized with 85% ethanol. A sterilized brass cork-borer (no. 9, 154 cm^2 in cross-sectional area) was used to prepare apple plugs, and a sterile knife was then used to cut apple plugs into disks (3 to 4 mm in thickness). Disks that contained or lacked skin were prepared from different fruits and from different areas of the same fruit. After preparation, apple disks were pooled together, and five disks from the pool were randomly selected, weighed, and used as a composite sample. Three composite samples that consisted of 15 disks were used in each experiment. The average (±SD) weight of the disk was 1.1 ± 0.2 g.

Inoculation of apple disks or intact fruits with bacterial suspensions. Inoculation was done by submerging apple disks or whole fruits in a bacterial suspension that contained a given concentration of *Salmonella* Chester for 30 s at room temperature. After immersion, apple disks or whole fruits were removed and air dried at room temperature for 10 min. Inoculated disks or fruits were rinsed twice in PBS, 50 ml for five disks and 500 ml for one fruit. Agitation was conducted in a Lab-Line Orbital Environ-Shaker (Lab-Line Instruments, Melrose, Ill.) at 200 rpm for 5 min. *Salmonella* Chester retained on the disks was determined by plating appropriately diluted tissue homogenates on BHIA-N medium. Homogenization was performed using a Seaward Stomacher (Model 400, Seaward Medial Ltd., London, England) at high speed for 2 min. To determine the distribution of attached *Salmonella* Chester on surfaces of three different parts (calyx, stem, and skin) of whole fruit, the calyx and stem cavities and adjacent tissue (15 mm radius from center axis) were excised from the fruit and the skin was peeled from the remaining surfaces of the fruits. A composite sample consisting of five whole fruits was used in each experiment. The calyx or stem portions excised from five fruits were pooled together and suspended in 100 ml of PBS for homogenization. The skin from the remaining surface of the five fruits was peeled, pooled, and suspended in 250 ml of PBS for homogenization. The number of *Salmonella* Chester in tissue homogenates was determined by placing the appropriately diluted tissue homogenate on BHIA-N medium.

Decontamination treatments. Four sanitizer solutions potentially useful for cleaning and decontamination treatments, including 6% (vol/vol) hydrogen peroxide *(3, 13)*, 2% (wt/vol) trisodium phosphate *(10, 22, 24)*, 0.36% (wt/vol) calcium hypochlorite *(3)*, and 1.76% (wt/vol) sodium hypochlorite *(3)*, were tested for their activities in inactivating attached *Salmonella* Chester on apple disks. The disks that containe *Salmonella* Chester were immersed in one of the four sanitizer solutions at room temperature for 5 min. After treatment, the disks were rinsed once with sterile water to remove a major portion of the residual sanitizer, and the number of viable bacteria that remained on the disks was determined. Samples treated with sterile water were used as controls. Similarly, intact fruits were immersed in a bacterial suspension that contained approximately 8.17 log CFU/ml of *Salmonella* Chester at room temperature for 10 min. After that, five inoculated fruits were submerged in the solution that contained 6% H_2O_2 alone or in the solution that contained 6% H_2O_2 plus 2% Tween 80 for 5 min. The additional five fruits were submerged in sterile water and used as control. Treated and untreated fruits were removed and rinsed once in sterile water to remove the residual sanitizer that remained on the fruits. The stem and calyx were excised from the fruit, the skin on the remaining fruits was peeled, and the number of bacteria on each part of the fruit was determined.

Statistical analysis. All experiments were done in triplicate, and a minimum of three samples were analyzed at each sample time. Analysis of variance and the Duncan multiple range test (SAS Institute, Inc., Cary, N.C.) were performed to determine significant differences on the logarithm (base 10) of bacterial population densities *(17)*. Significance was determined at the 0.05 level.

Scanning electron microscopy. Freshly prepared apple disks were immersed in a bacterial suspension that contained 9.17 log CFU/ml of *Salmonella* Chester. The stem and calyx parts excised from fruits that had previously been washed and surface sanitized with 85% ethanol were inoculated in the same way. Following two rinses with PBS, the disks and the stem and calyx parts were immersed for 2 h in 1% (wt/vol) glutaraldehyde, washed with PBS, postfixed in 2% (wt/vol) osmium tetroxide solution, and then dehydrated in a graded series of ethanol solutions and critical point dried with liquid carbon dioxide. The dry samples were mounted on aluminum stubs coated with a thin layer of gold by DC sputtering and examined by scanning electron microscopy.

RESULTS AND DISCUSSION

Effect of wash and agitation on removing *Salmonella* Chester from apple disks. Apple disks prepared as described above were uniform in size and shape. Because of their uniformity, apple disks served as a convenient and reliable laboratory model to study the attachment of human pathogens to plant tissues. To determine the effect of wash and agitation on removing bacteria from plant tissue, freshly prepared apple disks were immersed in a bacterial suspension that contained 7.17 log CFU/ml of *Salmonella* Chester for 30 s. The disks were then removed, air dried at room temperature for 10 min, and subjected to a series of washes with PBS. The numbers of *Salmonella* Chester that remained on the disks after each wash were determined as shown in Table 1. A total of 36 to 49% of *Salmonella* Chester retained on the disk after immersion in inoculum became firmly attached and could not be removed by two consecutive washes. An additional wash, even with agitation (200 rpm, 5 min), only removed a relatively small portion (4 to 6%) of bacteria from the disk. Results presented herein also show that agitation had no significant effect on removing the attached bacteria from the disks. The number of bacteria that remained on the disk that contained skin was 13 to 19% less than the number that remained on the disk that contained no skin, indicating that bacteria attached more efficiently and firmly to the surfaces of injured tissue than to the unbroken skin. Previously, Wei et al. *(23)* reported that chlorinated water was more effective in removing or inactivating *Salmonella* Montevideo on tomato skin than that on internal core tissue. It is possible that *Salmonella* Montevideo attaches more tightly to the surfaces of injured tomato tissue than to the unbroken skin and that bacteria remained on core tissue may be harder to remove by washing with chlorinated water. At present, little is known about the mechanism by which bacteria become at-

TABLE 1. *Effect of wash and agitation on removal of* Salmonella *Chester from fresh-cut apple disks*[a]

Treatment	Bacteria retained on disk with skin (log CFU/disk)[b]	Bacteria retained (%)	Bacteria retained on disks without skin (log CFU/disk)[b]	Bacteria retained (%)
No wash	6.46 ± 0.24 A[c]	100	6.61 ± 0.18 A	100
Wash once	6.14 ± 0.10 B	48	6.42 ± 0.14 B	64
Wash twice	6.02 ± 0.07 C	36	6.30 ± 0.06 C	49
Wash three times				
Without agitation	5.94 ± 0.16 D	30	6.26 ± 0.23 C	45
With agitation	5.89 ± 0.05 D	27	6.27 ± 0.12 C	46

[a] Apple disks that contained or lacked skin were immersed in a bacterial suspension that contained approximately 7.17 log CFU/ml of *Salmonella* Chester at room temperature for 30 s. Disks were removed, air dried at room temperature for 10 min, and then subjected to a series of washes using phosphate-buffered saline (PBS) for up to three times, five disks in 50 ml PBS each time. Agitation was done in a rotary shaker at 200 rpm for 5 min.

[b] These values represent the mean of three experiments ± SD; three composite samples consisting of 15 disks were used in each experiment.

[c] Mean values within a column followed by the same letter are not significantly different at the 0.05 level according to analysis of variance and the Duncan multiple range test.

tached to surfaces of food products. Physical forces such as London-van der Waals attraction, electrostatic attraction between two surfaces, and a net gain in entropy have been suggested *(8)*. Besides these forces, bacterial age and culture conditions may also play a role in the attachment *(7)*. Based on the results obtained from scanning electron microscopic observation as reported herein and previously *(8)*, retention of bacteria on injured tissue may be in part due to the physical entrapment of bacteria in the holes below the surface of injured tissue.

Number of *Salmonella* Chester attached as affected by inoculum density and immersion time. To determine the effect of inoculum density on attachment, apple disks were immersed in bacterial suspensions that contained increasing concentrations of *Salmonella* Chester, ranging from 5.17 to 9.17 log CFU/ml. Following two consecutive washes, the number of bacteria that remained on the disks was determined. Results (Table 2) show that the number of bacteria retained on the disks (log CFU/disk) was directly proportional to the concentration of bacteria in the suspension. The maximal attachment was observed with the disks that had been immersed in the suspension that contained 9.17 log CFU/ml of *Salmonella* Chester. Only a slight increase in the attachment was observed with the disks that had been immersed in suspensions that contained *Salmonella* Chester at levels higher than 9.17 log CFU/ml (data not shown). The bacterial density in the suspension is, therefore, a key factor in determining the number of bacteria attached to the disks. Previously, it has been shown that the number of bacteria attached to pork skin and beef carcasses was affected by the concentration of bacteria used for inoculation *(4)*. A direct correlation between the density of *Salmonella* Typhimurium used for inoculation and the number of bacteria attached to chicken skin has also been reported *(12)*.

TABLE 2. *Effect of inoculum level and immersion time on attachment of* Salmonella *Chester to apple disks*[a]

Immersion time (min)	Inoculum level (log CFU/ml)	No. bacteria recovered (log CFU/disk)[b]
0.5	5.17	4.29 ± 0.07
0.5	6.17	5.10 ± 0.10
0.5	7.17	6.35 ± 0.12
0.5	8.17	6.83 ± 0.04
0.5	9.17	7.14 ± 0.17
0.5	7.17	6.26 ± 0.13
2	7.17	6.37 ± 0.10
4	7.17	6.41 ± 0.06
8	7.17	6.43 ± 0.21
16	7.17	6.31 ± 0.09

[a] Freshly prepared apple disks were immersed in a bacterial suspension that contained approximately 7.17 log CFU/ml of *Salmonella* Chester for different periods. Disks were removed, air dried at room temperature for 10 min, and then rinsed twice in phosphate-buffered saline (PBS). Similarly, disks were immersed in bacterial suspensions that contained various concentrations of *Salmonella* Chester as indicated, and the numbers of bacteria retained on the disks were determined after air drying and two rinses in PBS.

[b] These values represent the mean of three experiments ± SD. In each experiment, three composite samples consisting of 15 disks were used.

To determine the effect of immersion time on attachment, apple disks were immersed in a bacterial suspension that contained approximately 7.17 log CFU/ml of *Salmonella* Chester for different periods, ranging from 30 s to 16 min. After two rinses with PBS, the number of bacteria that remained on the disks as a function of immersion time was determined. Table 2 shows that the total number of bacteria retained on the disks was not significantly affected by the duration of immersion time. Previously, Lillard *(12)* reported that attachment of *Salmonella* Typhimurium to chicken skin occurred within 15 s after submerging the chickens in a bacterial suspension. McMeekin and Thomas *(14)* also found no direct correlation between the number of bacteria retained on chickens and the time of immersion. Thus, the effect of inoculation time and inoculum density (as described above) on attachment of *Salmonella* to plant and animal food products appears to be similar.

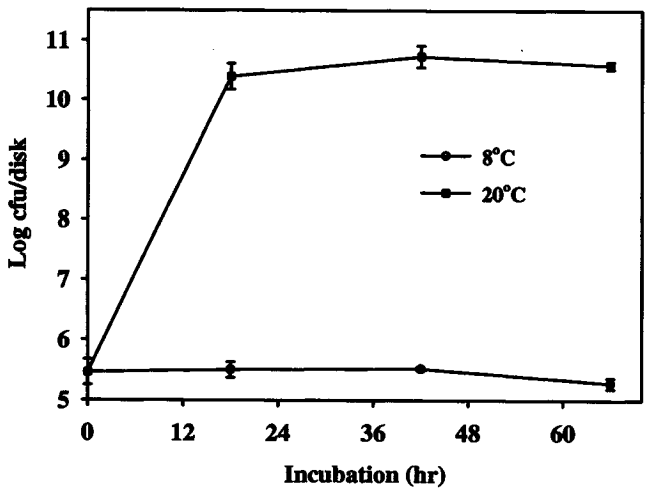


FIGURE 1. *Comparison of the growth of* Salmonella *Chester on apple disks at 8 and 20°C.*

Growth of *Salmonella* Chester on apple disks. To determine the growth of *Salmonella* Chester on apple disks (pH 4.1) at 8 and 20°C, apple disks that contained *Salmonella* Chester at an initial concentration of approximately 3.0×10^5 CFU/disk were incubated at either temperature, and the population of bacteria in the disk was determined daily for 4 days. Results (Fig. 1) show that *Salmonella* Chester failed to grow on apple disks at 8°C but grew well on the disks at 20°C. Such growth also might be expected to occur in stem punctures or bruises occasionally seen in fresh apples *(16)*. Acid tolerance appears to be common among *Salmonella* serovars. Growth of *Salmonella* Typhimurium in apple juice (pH 3.4 to 3.9) *(9)* and growth of *Salmonella* Montevideo in tomato core tissue (pH 4.1 to 4.3) *(23, 25)* have been previously reported. Another foodborne pathogen, *E. coli* O157:H7, is also able to grow on injured apple tissue *(11)*. All these studies indicate that it is important to store apple fruits destined for unpasteurized juice production or for fresh-cut products at low temperatures (8°C or below) to suppress the growth of *Salmonella* and other foodborne pathogens.

Stem and calyx cavity areas are the principal sites for attachment. To determine the effect of washing on removing bacteria from surfaces of three different parts of whole fruit (calyx, stem, and skin), 10 intact fruits were immersed in a bacterial suspension that contained approximately 7.17 log CFU/ml of *Salmonella* Chester and then air dried at room temperature for 10 min. Five of the 10 fruits were washed twice with PBS to determine the number of bacteria retained on fruits after washing. The other five fruits that received no washing were used to determine the number of bacteria retained on fruits before washing. Results (Table 3) show that most bacteria retained on fruits after washing were found either on the stem (53%) or calyx (41%) cavity area, and a relatively smaller proportion (6%) was found on the skin area. This result indicated that *Salmonella* Chester attached more efficiently and firmly to surfaces in stem and calyx areas than to the unbroken skin. Surfaces in the stem and calyx cavities and injured tissue (as described above) appeared to be the principal sites of bacterial attachment. Firm attachment of bacteria to these sites represents a major obstacle for developing effective methods for eliminating *Salmonella* Chester from contaminated fruits. It has been shown previously that, when raw tomato fruits were immersed in a *Salmonella* Montevideo suspension, more bacteria were retained on stem scars and core tissues than on tomato skin *(23)*. The morphological and biochemical basis for the greater attachment of bacteria to surfaces of injured fruit tissue than to unbroken skin is currently unclear. Seo and Frank *(18)* speculated that *E. coli* O157:H7 was entrapped within the stomata below the surface of stomata and cut edge of lettuce leaves. So far, there is no direct evidence that bacteria entrapped in the holes below the surface of injured tissue are unremovable by washing. It is also unclear if the poor attachment of bacteria to fruit skin is due to the smooth surface or waxy coating of the skin or due to the lack of specific cellular components required for attachment.

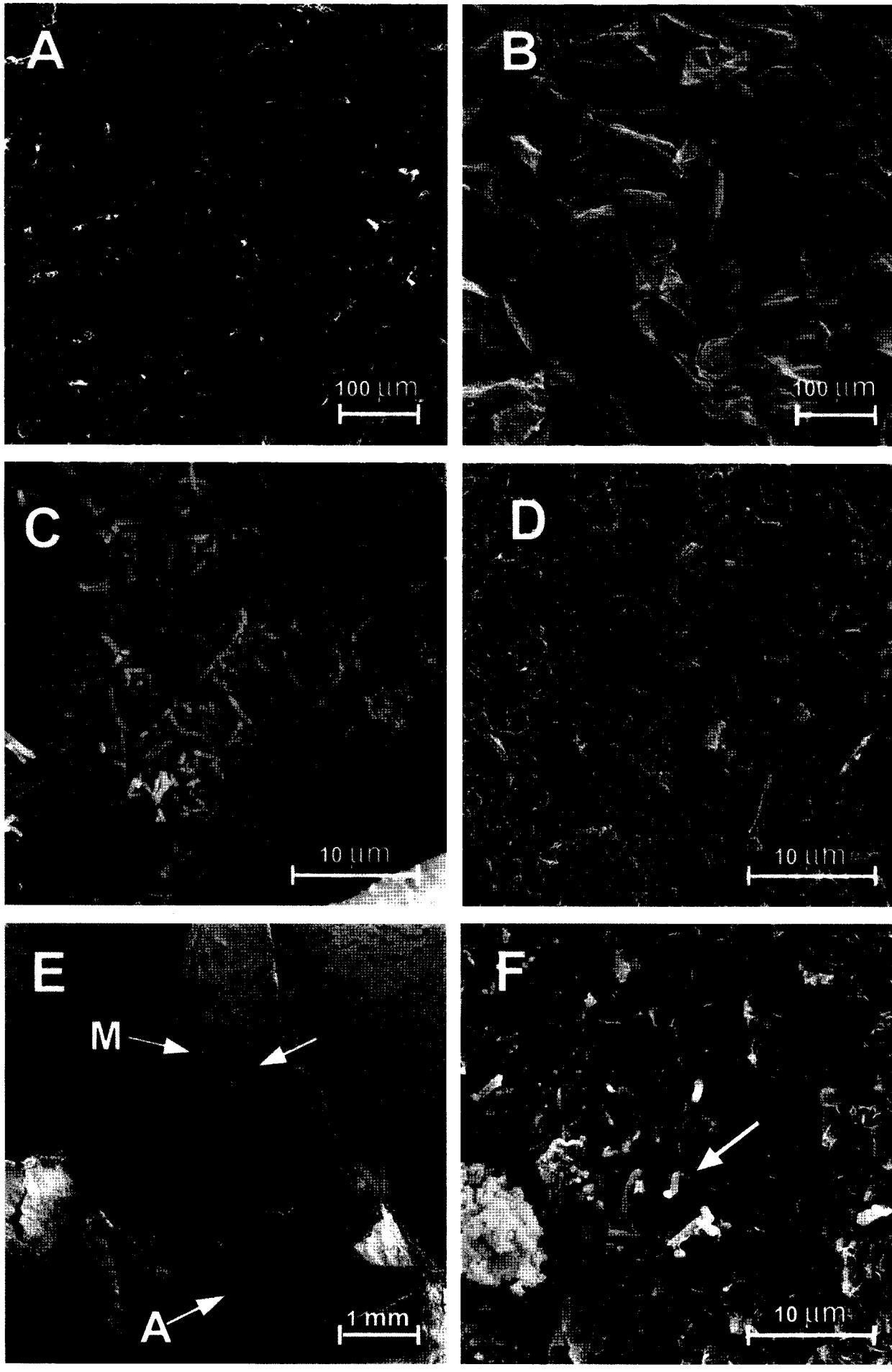
Topographical structure of apple fruit related to bacterial attachment. Topographical structure of apple skin and injured (cut) fleshy tissue was examined using a scanning electron microscope. Figure 2A shows the relatively smooth surface of apple skin. The rough surfaces of injured (cut) tissues are shown in Figure 2B, and numerous holes below the surface of injured tissue were observed. In general, attachment of *Salmonella* Chester was detected mainly on the surfaces of injured tissue and stem and calyx

TABLE 3. *Distribution of attached* Salmonella *Chester on surfaces of three different parts (stem, calyx, and skin) of apple fruits*[a]

Location of bacteria	Total no. of bacteria before washing (mean log CFU/apple)[b]	Total no. of bacteria after washing (mean log CFU/apple)	Bacteria retained after washing (%)	Distribution of bacteria on each part of fruit (%)
Stem cavity	5.57 ± 0.13	5.28 ± 0.09	51	53
Calyx cavity	5.65 ± 0.17	5.18 ± 0.21	33	41
Skin peel	5.58 ± 0.23	4.28 ± 0.31	6	6

[a] Apple fruits were immersed in a bacterial suspension that contained approximately 7.17 log CFU/ml of *Salmonella* Chester for 30 s at room temperature. Fruits were removed and air dried at room temperature for 10 min. The stem and calyx cavity regions were removed separately from each fruit and the remaining skin on the fruit was peeled as described in the "Materials and Methods" section.

[b] The value represents an average of three experiments ± SD. A composite sample, consisting of five fruits, was used to determine the number of bacteria before and after washing. The average weight of fruit was 610 g per fruit.


A
100 μm
B
100 μm
C
10 μm
D
10 μm
E
M
A
1 mm
F
10 μm

parts but rarely on the unbroken skin. Microcolonies formed on injured tissue (Fig. 2C), stem (Fig. 2D), and calyx (not shown) were incubated at 20°C for 18 h. Occasionally, a biofilm structure on the surface of inoculated calyx or stem (Fig. 2D) was observed, but the nature and origin of this biofilm structure was not determined. The surfaces of stem and calyx cavities were naturally and frequently contaminated with fungal mycelia (Fig. 2E). The rough surface of calyx served as the site for the attachment of *Salmonella* Chester (Fig. 2F). However, it is not clear if presence of fungal mycylia on the surfaces of stem and calyx affects the attachment. The greater attachment of *Salmonella* Chester to injured tissue and stem and calyx cavitites than to unbroken skin is possibly due to their difference in topographical structure and specific physicochemical properties.

Partial resistance of attached bacteria to sanitizer treatments. To determine the efficacy of four chemical sanitizers (6% hydrogen peroxide, 2% trisodium phosphate, 0.36% calcium hypochlorite, and 1.76% sodium hypochlorite) in inactivating *Salmonella* Chester attached to apple disks, the disks that contained attached *Salmonella* Chester were treated with one of the four sanitizers at room temperature for 5 min. Viable bacteria that remained on the disks after treatment were determined. Results (Table 4) show that all four sanitizers tested were only partially effective in inactivating *Salmonella* Chester attached to apple disks. The population of *Salmonella* Chester on apple disks was reduced from 6.42 log CFU/disk before dipping in one of the four sanitizers to 5.55 to 5.11 log CFU/disk. Hydrogen peroxide, which had been previously recommended for use in poultry chill water *(13)*, was the most effective among the four sanitizers tested. It reduced the bacterial population on apple disks by 1.31 logs, whereas the other three sanitizers reduced the population by 0.87 to 1.10 logs. Despite its effectiveness, hydrogen peroxide treatment of apple slices resulted in the organoleptic change of the food. Although calcium hypochlorite (0.36%, pH 6.8) was generally more effective in reducing the population of *Salmonella* Chester on apple disks than sodium hypochlorite (1.76%, pH 6.8) and trisodium polyphosphate (2%, pH 12.3), there was no significant difference in their effectiveness between sodium hypochlorite and trisodium polyphosphate (Table 4).

Because of its effectiveness against *Salmonella* Chester on apple disks and against *E. coli* on apple fruits *(16)*, hydrogen peroxide was subsequently tested for its in vivo activity against *Salmonella* Chester on three different parts of the fruit (stem, calyx, and skin). Inoculated whole fruits were dipped in the solution that contained 6% hydrogen peroxide or 6% hydrogen peroxide plus 2% Tween 80 for 5 min. The fruits were then removed and washed once in sterile water, and the numbers of bacteria on each part of fruit were determined. Results (Table 5) show that a total of 7.13 log units of bacteria were recovered from the entire fruit after immersing the fruit in a suspension that contained 8.17 log CFU/ml of *Salmonella* Chester. The number of *Salmonella* Chester on the fruit was reduced to 5.29 and 5.18 log/fruit after treating the fruit with hydrogen peroxide with or without added Tween 80. Addition of Tween 80 in hydrogen peroxide solution had no significant effect on removing bacteria from contaminated fruits. However, the consistent difference in surviving *Salmonella* Chester on disks treated with hydrogen peroxide with or without Tween 80 suggests that addition of Tween 80 or some other surfactant might be worthy of further investigations. Addition of 5% Tween 80 in trisodium phosphate solution has been shown to enhance the removal of *Salmonella* from chicken skin *(10)*. In this study, we found that the hydrogen peroxide treatment reduced the number of *Salmonella* Chester on skin by 3 to 4 logs/fruit and the number of bacteria on stem and calyx by 1 to 2 logs/fruit, yielding an

TABLE 4. *Effectiveness of various sanitizers on inactivating* Salmonella *Chester attached to the surface of apple disks*[a]

Treatment	No. of bacteria remained on disks (log CFU/disk)[b]	Bacteria survived (%)	Log CFU reduction
H_2O (control)	6.42 ± 0.21 A[c]	100	0
CaOCl (0.36%) pH 6.8	5.32 ± 0.16 B	8	1.10
NaOCl (1.76%) pH 6.8	5.55 ± 0.04 C	13	0.87
TSP (2%) pH 12.3	5.47 ± 0.07 C	11	0.95
H_2O_2 (6%)	5.11 ± 0.13 D	5	1.31

[a] Apple disks were immersed in a bacterial suspension that contained approximately 7.17 log CFU/ml of *Salmonella* Chester at room temperature for 30 s. Disks were removed and air dried at room temperature for 10 min. After rinsing twice in phosphate-buffered saline, disks were immersed in a sanitizer solution for 5 min. Treated disks were then removed and washed once in sterile water to remove the residual sanitizer that remained on the disk. TSP, trisodium phosphate; CaOCl, calcium hypochlorite; NaOCl, sodium hypochlorite.

[b] The value represents the mean of three independent experiments. Three composite samples consisting of 15 disks were used in each experiment.

[c] Means within the column followed by the same letter are not significantly different at the 0.05 level based on variance analysis and the Duncan multiple range test.

←

FIGURE 2. *Scanning electron micrographs of* Salmonella *Chester–inoculated apple disk and calyx and stem parts of fruit. (A) Topographical structure of apple skin (original magnification ×15). (B) Surface structure of injured or cut tissue (original magnification ×15). (C) Attachment of* Salmonella *Chester on surfaces of injured tissue (original magnification ×2,500). (D) Formation of microcolonies on surfaces of stem after incubation at 20°C for 18 h (original magnification ×2,500). (E) Topographical structure of calyx inoculated with* Salmonella *Chester; M arrow, naturally contaminated fungal mycelium; A arrow, residual anther; and arrow alone (without letter), the area enlarged in figure F (original magnification ×15). (F) Enlarged area of figure E, showing the attachment of* Salmonella *Chester on calyx; arrow points to the attached bacteria (original magnification ×2,500).*

TABLE 5. *Effectiveness of hydrogen peroxide on inactivating* Salmonella *Chester attached to three different parts of apple fruits (stem, calyx, and skin) as affected by the presence or absence of Tween 80*[a]

Treatment	No. of bacteria (log CFU/fruit) remaining[b]			
	Stem cavity	Calyx cavity	Skin peel	Total
No wash	6.75 ± 0.05 A[c]	6.49 ± 0.21 A	6.67 ± 0.17 A	7.13 ± 0.04 A
Washed with H_2O_2	4.95 ± 0.12 B	4.97 ± 0.23 B	3.00 ± 0.04 B	5.29 ± 0.12 B
Washed with H_2O_2 and 2% Tween 80	4.86 ± 0.09 B	4.89 ± 0.12 B	2.84 ± 0.08 B	5.18 ± 0.20 B
Log CFU reduction	1.89	1.60	3.83	1.95

[a] Five inoculated fruits were submerged in 6% H_2O_2 or in 6% H_2O_2 plus 2% Tween 80 for 5 min. Five fruits submerged in sterile water were used as control. After treatment, fruits were removed and rinsed once in sterile water to remove the residual sanitizer. The stem and calyx cavities were excised from each fruit, and the skin on the remaining fruit was peeled. The number of bacteria on each part of fruit was determined as described in the "Materials and Methods" section.

[b] The value represents the mean of three experiments ± SD. Total indicates the combined number of bacteria on three different parts of fruit. Log CFU reduction indicates the difference in the number of bacteria before and after treatment with 6% H_2O_2 plus 2% Tween 80.

[c] Means within each column followed by the same letter are not significantly different at the 0.05 level according to analysis of variance and the Duncan multiple range test.

overall reduction of only 2 logs/fruit. However, the Food and Drug Administration is calling for a 5-log reduction in the population of *E. coli* O157:H7 in unpasteurized apple cider. A small portion of bacteria attached to stem and calyx was found either resistant to or protected from the sanitizer treatment. Failure of sanitizers to completely inactivate *Salmonella* Chester on apple fruits was likely due to the firm attachment of bacteria on stem and calyx and partial resistance of attached bacteria to sanitizer treatment. Tamblyn and Conner *(19)* reported that *Salmonella* Typhimurium attached to chicken skin exhibited tolerance to organic acid treatment. However, it has been suggested that failure of chlorinated water to completely eliminate *Salmonella* Montevideo from tomato tissue was due to the presence of bacteria within the tissue where they are inaccessible by chemical sanitizers *(3)*. Further investigation on the mechanism as to how bacteria become attached to apple fruits and how attached bacteria become resistant to or are protected from the sanitizer treatment would lead to the development of more effective methods for cleaning and decontaminating apple fruits destined for juice production, fresh-cut products, or fresh consumption.

ACKNOWLEDGMENT

We thank Larry G. Revear and Douglas S. Soroka for technical assistance and Peter H. Cooke for conducting scanning electron microscopic examinations. We also thank Dr. Kevin B. Hicks for critical review and suggestions during the preparation of the manuscript.

REFERENCES

1. Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barett, J. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. JAMA 269:2217–2220.
2. Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. J. Food Prot. 59:204–216.
3. Beuchat, L. R. 1997. Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. Int. J. Food Microbiol. 34:329–333.
4. Butler, J. L., J. C. Stewart, C. Vanderzant, Z. L. Carpenter, and G. C. Smith. 1979. Attachment of microorganisms to pork skin and surfaces of beef and lamb carcasses. J. Food Prot. 42:401–406.
5. Centers for Disease Control and Prevention. 1975. *Salmonella* outbreak traced to a commercial apple cider: New Jersey. Morbid. Mortal. Weekly Rep. 24:87–88.
6. Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* infection and cryptosporidiosis associated with drinking unpasteurized apple cider: Connecticut and New York. October, 1996. Morbid. Mortal. Weekly Rep. 46:4–8.
7. Dickson, J. S., and M. Koohmaraie. 1989. Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. Appl. Environ. Microbiol. 55:832–836.
8. Firstenberg-Eden, R., S. Notermans, F. Thiel, S. Henstra, and E. H. Kampelmacher. 1979. Scanning electron microscopic investigations into attachment of bacteria to teats of cow. J. Food Prot. 42:305–309.
9. Goverd, K. A., F. W. Beech, R. P. Hobbs, and R. Shannon. 1979. The occurrence and survival of coliforms and Salmonellas in apple juice and cider. J. Appl. Microbiol. 46:521–530.
10. Hwang, C.-A., and L. R. Beuchat. 1995. Efficacy of selected chemicals for killing pathogenic and spoilage microorganisms on chicken skin. J. Food Prot. 58:19–23.
11. Janisiewicz, W. J., W. S. Conway, M. W. Brown, G. M. Sapers, P. Fratamico, and R. L. Buchanan. 1999. Fate of *Escherichia coli* O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. Appl. Environ. Microbiol. 65:1–5.
12. Lillard, H. S. 1988. Effect of surfactant or changes in ionic strength on the attachment of *Salmonella typhimurium* to poultry skin and muscle. J. Food Prot. 53:727–730.
13. Lillard, H. S., and J. E. Thomson. 1983. Efficacy of hydrogen peroxide as a bactericide in poultry chiller water. J. Food Sci. 48:125–126.
14. McMeekin, T. A., and C. J. Thomas. 1978. Retention of bacteria on chicken skin after immersion in bacterial suspensions. J. Appl. Microbiol. 45:383–387.
15. Notermans, S., and E. H. Kampelmacher. 1974. Attachment of some bacterial strains to the skin of broiler chickens. Br. Poult. Sci. 15: 573–585.
16. Sapers, G. M., R. L. Miller, and A. M. Mattrazzo. Effectiveness of sanitizing agents in inactivating *Escherichia coli* in golden delicious apples. J. Food Sci., in press.
17. SAS Institute. 1985. SAS user's guide: statistics, version 5. SAS Institute, Cary, NC.
18. Seo, K. H., and J. F. Frank. 1999. Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. J. Food Prot. 62:3–9.
19. Tamblyn, K. C., and D. E. Conner. 1997. Bactericidal activity of

organic acids against *Salmonella typhimurium* attached to broiler chicken skin. J. Food Prot. 60:629–633.

20. Tauxe, R., H. Kruse, C. Hedberg, M. Potter, J. Madden, and K. Wachsmuth. 1997. Microbial hazards and emerging issues associated with produce: a preliminary report to the National Advisory Committee on Microbiologic Criteria for Foods. J. Food Prot. 60:1400–1408.
21. Wachtel, M. R., S. M. Leffel, and R. E. Mandrell. 1999. Attachment of enterohemorrhagic *Escherichia coli* O157:H7 to lettuce leaf surfaces, p. 525. Abstr. 96th Gen. Meet. Am. Soc. Microbiol. 1999. American Society for Microbiology, Washington, D.C.
22. Wang, W. C., Y. Li, M. F. Slavik, and H. Xiong. 1997. Tridodium phosphate and cetylpyridinium chloride spraying on chicken skin to reduce attached *Salmonella typhimurium*. J. Food Prot. 69:992–994.
23. Wei, C. I., T. S. Huang, J. M. Kim, W. F. Lin, M. L. Tamplin, and J. A. Bartz. 1995. Growth and survival of *Salmonella montevideo* on tomatoes and disinfection with chlorinated water. J. Food Prot. 58:829–836.
24. Zhuang, R. Y., and L. R. Beuchat. 1996. Effectiveness of trisodium phosphate for killing *Salmonella montevideo* on tomatoes. Lett. Appl. Microbiol. 22:97–100.
25. Zhuang, R. Y., L. R. Beuchat, and F. J. Angulo. 1995. Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment with chlorine. Appl. Environ. Microbiol. 61: 2127–2131.